CLAIMS

What is claimed is:

1. A method for the optimization of the production of a genetic end product comprising:

a) providing a multiplicity of integration cassettes, each cassette comprising:
   (i) a nucleic acid integration fragment;
   (ii) a selectable marker bounded by specific recombinase sites responsive to a recombinase;
   (iii) homology arms having homology to different portions of a donor cell chromosome;

b) transforming at least one donor cell with the integration cassettes of (a) for its chromosomal integration;

c) infecting the transformed donor cell of (b) with a phage wherein the phage replicates and the donor cell is lysed;

d) isolating phage released by the lysis of the donor cell of (c);

e) mixing isolated phage released by the lysis the of donor cells of (c) carrying different integration cassettes of (a);

f) infecting a recipient cell with the mixture of the isolated phage of (e) wherein the integration cassettes each integrate into the recipient cell chromosome at the point of homology to the homology arms to generate a transduced recipient cell;

g) selecting transduced recipient cells on the basis of the selectable marker;

h) screening the recipient cell of (g) for the highest level of the genetic end product to identify a first overproducing strain;

i) activating a recombinase in the first over producing strain of (h) which excises the selectable marker from the chromosomally integrated integration cassette;

j) infecting the first over producing strain of (i) with the mixture of the isolated phage of (e) wherein the integration cassettes each integrate into the recipient cell chromosome at the point of homology on the homology arms;

k) screening the infected first over producing strain of (j) for the highest level of the genetic end product to identify a second overproducing strain; and

l) comparing the levels of genetic end product produced by the first and second over producing strains whereby the production of the genetic end product is optimized.

2. A method according to Claim 1 wherein the nucleic acid integration fragment is selected from the group consisting of; a promoter, a gene, a mutated gene, a disrupted gene, a coding region, and a non-coding region.

3. A method according to Claim 1 wherein the integration cassette has the general structure 5'-RR1-RS-SM-RS-Y-RR2-3' wherein:

(i) RR1 is a first homology arm;

(ii) RS is a recombination site responsive to a site-specific recombinase;

(iii) SM is a DNA fragment encoding a selectable marker;

(iv) Y is a first expressible DNA fragment; and

(v) RR2 is a second homology arm.

4. A method according to Claim 1 wherein the donor cell and the recipient cell are bacterial cells.

5. A method according to Claim 4 wherein the donor cell and the recipient cell are selected from the group consisting of *Agrobacterium, Erythrobacter, Chlorobium, Chromatium, Flavobacterium, Cytophaga, Rhodobacter, Rhodococcus, Streptomyces, Brevibacterium, Corynebacteria, Mycobacterium, Deinococcus, Paracoccus, Escherichia, Bacillus*, *Myxococcus*, *Salmonella, Yersinia, Erwinia, Pantoea, Pseudomonas, Sphingomonas, Methylomonas, Methylobacter, Methylococcus, Methylosinus, Methylomicrobium, Methylocystis, Alcaligenes, Synechocystis, Synechococcus, Anabaena, Thiobacillus, Methanobacterium, Klebsiella,* and *Myxococcus*.

6. A method according to Claim 1 wherein the phage is selected from the group consisting of P1, P2, lambda, ϕ80, ϕ3538, T1, T4, P22, P22 derivatives, ES18, Felix "o", P1-CmCs, Ffm, PY20, Mx4, Mx8, PBS-1, PMB-1, and PBT-1.

7. A method according to Claim 1 wherein the genetic end product is selected from the group consisting of, isoprenoids, terpenoids, tetrapyrroles, polyketides, vitamins, amino acids, fatty acids, proteins,

nucleic acids, carbohydrates, antimicrobial agents, anticancer agents and biological metabolites.

8. A method according to Claim 1 wherein steps (f) – (l) are repeated to improve the optimization, wherein the recipient cell of (f) is the second and subsequent over producing strains of (k).

9. A method according to Claim 1 wherein recombinase and recombinase site system is selected from the group consisting of Cre-*lox*, FLP/*FRT*, R/RS, Gin/gix, Xer/*dif*, Int/*att*, a pSR1 system, a cer system, and a fim system.

10. A method according to Claim 1 wherein the recipient cell is an *E. coli*.

11. A method according to Claim 1 wherein the homology arms are from about 5 bases to about 100 bases.

12. A method according to Claim 1 wherein the homology arms have at least 90% identity to a portion of the donor cell chromosome.

13. A method for the optimization of the production of a genetic end product comprising:

a) providing a multiplicity of integration cassettes, each cassette comprising:
   (i) a promoter;
   (ii) a selectable marker bounded by specific recombinase sites responsive to a recombinase;
   (iii) regions of homology to different portions of a P1 donor cell chromosome;

b) transforming at least one donor cell with the integration cassette of (a) for its chromosomal integration;

c) infecting the transformed donor cell of (b) with a P1 phage wherein the phage replicates and the donor cell is lysed;

d) isolating phage released by the lysis of the donor cell of (c);

e) mixing equal number of isolating phage released by the lysis of a set of donor cells of (c) carrying different integration cassettes of (a);

f) infecting a recipient cell with the mixture of the isolated phage of (e) wherein the integration cassettes each integrate into the recipient cell chromosome at the point of homology to the homology arms;

g) selecting transduced recipient cells on the basis of the selectable marker;

h) screening the recipient cell of (f) for the highest level of the genetic end product to identify a first overproducing strain;

i) activating a recombinase in the first over producing strain of (h) which excises the selectable marker from the chromosomally integrated integration cassette;

j) infecting the first over producing strain of (i) with the mixture of the isolated phage of (e) wherein the integration cassettes each integrate into the recipient cell chromosome at the point of homology on the homology arms;

k) screening the first over producing strain of (j) for the highest level of the genetic end product to identify a second overproducing strain; and

i) comparing the levels of genetic end product produced by the first and second over producing strains whereby the production of the genetic end product is optimized.

14. A method according to Claim 13 wherein the promoter regions are derived from a cell other than the donor cell or recipient cell.

15. A method according to Claim 13 wherein the promoter is selected from the group consisting of *lac*, *ara*, *tet*, *trp*, λP_L, λP_R, *T7*, *tac*, P_{T5}, and *trc*.

16. A method according to Claim 13 wherein the promoter is P_{T5}.

17. A method according to Claim 13 wherein the donor cell and recipient cell have the genes that comprise the isoprenoid biosynthetic pathway.

18. A method according to Claim 17 wherein the integration cassette integrates into the recipient chromosome so as to operably link the promoter and a gene of the isoprenoid biosynthetic pathway.

19. A method according to Claim 18 wherein the genes of the isoprenoid biosynthetic pathway are selected from the group consisting of *dxs*, *dxr*, *ygbP*, *ychB*, *ygbB*, *idi*, *ispA*, *lytB*, *gcpE*, *ispA*, *ispB*, *crtE*, *crtY*, *crtI*, *crtB*, *crtX*, *crtW*, *crtO*, *crtR*, and *crtZ*.

20. A method according to Claim 18 wherein the genetic end product is a carotenoid selected from the group consisting of antheraxanthin, adonixanthin, astaxanthin, canthaxanthin, capsorubrin, β-

cryptoxanthin, didehydrolycopene, didehydrolycopene, β-carotene, ζ–carotene, δ–carotene, γ–carotene, keto–γ–carotene, ψ–carotene, ε–carotene, β,ψ–carotene, torulene, echinenone, gamma-carotene, zeta-carotene, alpha-cryptoxanthin, diatoxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol, isorenieratene, β-isorenieratene lactucaxanthin, lutein, lycopene, neoxanthin, neurosporene, hydroxyneurosporene, peridinin, phytoene, rhodopin, rhodopin glucoside, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, uriolide, uriolide acetate, violaxanthin, zeaxanthin-β-diglucoside, zeaxanthin, and C30-carotenoids.